The role of **micro**plastic in the **s**pread and persis**t**ence of antibioti**c** resistance under p**h**a**r**maceuticals and h**ea**vy me**t**al pre**s**sures in the riverine eco**s**ystem

Acronym: MICROTHREATS

According to the World Health Organization (WHO) report, the spread of antimicrobial resistance (AMR) has reached alarmingly high levels, making it one of the greatest public health challenges of the 21st century. Infections caused by antibiotic-resistant bacteria (ARB) are becoming an increasing threat, resulting in 1.27 million deaths worldwide in 2019. Estimations indicate that this number could rise to 10 million by 2050 if effective actions are not taken. Particularly dangerous are the so-called "last-resort" antibiotic-resistant strains, which do not respond to available drugs. The lack of an effective response to this phenomenon leads to the threat of a post-antibiotic era, where even common infections could become fatal.

Human activity leads to global changes, and one of the negative consequences of the development of civilization and the growth of the human population is environmental pollution, including surface waters. Microplastics (MPs), heavy metals, and pharmaceuticals from industry, agriculture, and wastewater are factors that may promote the spread of ARB and antibiotic resistance genes (ARGs) in the aquatic environment. According to the 2022 WHO report, MPs have been recognized as a serious threat to both the environment and public health, including contributing to the spread of antibiotic-resistant pathogens. Understanding the role that MPs play in the spread of antibiotic resistance in aquatic ecosystems is becoming crucial.

The aim of this project is to assess the impact of MPs on the increase of the aquatic resistome gene pool (the total collection of ARGs) in the presence of pharmaceuticals and heavy metals in the aquatic environment of the Oder River, which flows through three Central European countries (Czech Republic, Germany, and Poland). The research will focus on determining how human activity-related pollutants (MPs, heavy metals, and pharmaceuticals) contribute to the emergence and spread of ARB and ARGs in the river's waters, particularly in the context of the ecological disaster caused by climate change and pollution. The samples will be collected along an anthropogenically-influenced gradient, from upstream through midstream to downstream as well as at the mouth of the Odra River into the Baltic Sea.

The project will include:

- Determining the frequency and types of ARGs in bacteria with significant antibiotic resistance mechanisms isolated from MPs and surrounding waters and sediments.
- Quantitative determination of ARGs and integrons in the metagenome (total DNA) and plasmidome (total collection of plasmids) from MPs, waters, and sediments.
- Analysis of the diversity of ARGs and integrons in the metagenome and plasmidome from MPs and surrounding waters and sediments.
- Determining the composition of bacterial populations on MPs and in surrounding waters and sediments.
- Determining the frequency of ARGs and integrons located on mobile genetic elements.
- Measuring the levels and diversity of pharmaceuticals and heavy metals in waters and sediments.

These studies will help to develop strategies to address environmental and public health threats related to the spread of antibiotic resistance. To date, no research has been conducted in Poland on antibiotic resistance in rivers in relation to pollution caused by human activity (MPs, pharmaceuticals, heavy metals). This project is pioneering, especially in the context of studying resistance in the Oder River. These studies also align with the WHO's efforts and the Global Antimicrobial Resistance Surveillance System (GLASS), which aims to develop an effective strategy for monitoring and assessing unknown pathways of antimicrobial resistance and pathogen spread in the environment. Understanding the role of MPs in this process is critical to the development of effective actions to mitigate the impact of this phenomenon on public health and the environment. Given the global spread of MPs in aquatic environments, the proposed study will help determine whether the influence of MPs on the spread of ARB, ARGs, and integrons may pose a future threat to public health, and how the presence of pharmaceuticals and heavy metals influences the dynamics of this process.